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Folic Acid Supplementation Reduced Depression Score in Young Japanese Women with Depression-susceptible Genetic Polymorphisms

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Abstract

Background: Observational studies have shown that low folate status induces depression. Here we studied the effects of folic acid supplementation on depression scores of Japanese women with depression-susceptible gene polymorphisms: methylenetetrahydrofolate reductase (*MTHFR*), serotonin transporter (5-*HTT*), and dopamine D4 receptor (*DRD4*).

Design: This was a 16-wk randomized, double-blind, crossover study of a group of Japanese women. Forty-six women (mean age 20 years; range: 18-26) were included in the study. First it was determined if the subjects had one of the following depression-susceptible gene polymorphisms: *MTHFR*, 5-*HTT*, or *DRD4*. The subjects then consumed bread with or without folic acid (200 μg/d) during two 4-wk diet periods separated by an 8-wk washout period. Serum folate and serum total homocysteine (tHcy) concentrations were measured before and after each intervention. Depressive symptoms using the Center for Epidemiologic Studies Depression Scale (CES-D) were determined.

Results: In the folic acid period, serum folate concentration increased from 14.2 nmol/L (median, 25th-75th percentiles: 10.6-17.5) to 23.1 nmol/L (16.4-27.8 $p < 0.001$); tHcy was reduced from 6.6 μmol/L (5.6-7.5) to 5.7 μmol/L (4.8-6.6, $p < 0.001$) and CES-D score decreased from 10.0 (3.0-13.0) to 5.0 (2.0-10.0, $p < 0.001$). In the control period, these changes were either insignificant or reversed. CES-D score significantly decreased by supplementing with folic acid in subjects with depression-susceptible genotypes common among Japanese, namely homozygotes of the "S" allele of 5-*HTT* and the "4" allele of *DRD4*.

Keywords: Folic acid; Depression; Homocysteine; Serotonin transporter; Polymorphism

List of abbreviations: 5-HTT-Serotonin transporter gene; BDHQ-Brief-type self-administered diet history questionnaire; CES-D-Center for Epidemiologic Studies Depression Scale; DFE-Dietary folate equivalents, DRD4-Dopamine D4 receptor gene; MRI-Magnetic resonance imaging; MTHFR- Methylenetetrahydrofolate reductase gene; RDA-Recommended dietary allowances; tHcy-Serum total homocysteine; VNTR-variable number tandem repeat

Introduction

Folate is required in the brain for the synthesis of monoamine neurotransmitters, and numerous observational studies have suggested that there is an association between folate deficiency and depression [1]. Specifically, high serum total homocysteine (tHcy) and the *T/T* homozygote variant of the C677T polymorphism in the methylenetetrahydrofolate reductase gene (*MTHFR*) have been associated with depression [1,2]. As pointed out by Bottiglieri [1], there are surprisingly few intervention studies using folic acid supplement in subjects with depression to date. The causal relationship between depression and folate deficiency in case-control studies cannot be determined. Thus, a randomized controlled study is needed, especially for Japanese with genetic polymorphisms that increase one's susceptibility to depression.

The *T/T* homozygote genotype of *MTHFR*, occurring in 17.3% of Japanese [3] and 4-12% of Caucasians [1], has been associated with significantly lower serum folate and higher tHcy than other genotypes [1,3]. We selected *MTHFR* because the odds ratio for depression for *T/T* homozygotes versus the wild type (C/C homozygote) is 2.8 ($p=0.005$) in Japanese [4], which is higher than in other ethnic groups [1], as compared in the HuGE review [5]. A polymorphism (short allele = S and long allele = L) in the 5'regulatory promoter region of the serotonin transporter gene (5-*HTT*) has been found to moderate the influence of stressful life events on depression [6]. Subjects who are *S/S* homozygotes of 5-*HTT* are three times more likely to be depressed in response to stressful life events than subjects who are *L/L* homozygotes [6].

We have found that the variant frequency of S/S homozygotes of 5-*HTT* was 69.3% in Japanese [7], which was 4.3-fold higher than the prevalence in populations of Caucasians (16%) [8]. In addition, having short alleles of the dopamine D4 receptor gene (*DRD4*) 48-base-pair-repeat polymorphism has been shown to be a risk factor for depression [9]. We have also found that the variant frequency of the *4/4* homozygote was 62.9% among Japanese [7], which was higher than the prevalence in Caucasians [10]. Nanri et al. [11] analyzed cross-sectional data for employees, and depressive symptoms were assessed using the CES-D scale [12]. The lowest quartile of serum folate was associated with increased depressive symptoms (odds ratio=0.51; trend p=0.03) in men [11]. Thus, it was suggested folate be used in the treatment and prevention of depressive symptoms [1,13,14].

In this report, we measured serum folate and tHcy concentrations, and the CES-D score of Japanese women with the three genetic polymorphisms, *MTHFR*, 5-*HTT*, and *DRD4*, in a randomized double-blind controlled study using folic acid-fortified and control bread.

Methods

Study design

Young healthy Japanese women from one university (n=46; mean age: 20 years, range: 18-26) were recruited, whose blood chemistry profiles were normal. None of the subjects were using vitamin supplements. Subject baseline characteristics are summarized in Table 1. The study was carried out in accordance with the instructions of the Declaration of Helsinki, study procedures were approved by the Kagawa Nutrition University Human Subjects and Genome Ethics Committee (No. G-50), and written informed consent was obtained from all subjects.

	Group A n=24	Group B n=22	Total n=46
Age, y	20 (19-21)	20 (19-21)	20 (19-21)
Weight, kg	54.0 (50.0-58.0)	52.6 (48.4-52.6)	52.9 (48.9-58.0)
BMI, kg/m2	21.4 (19.4-22.1)	20.8 (19.6-22.5)	21.1 (19.5-22.1)
Serum tHcy, μmol/L	6.4 (5.4-7.5)	7.1 (6.4-9.0)	6.8 (5.8-7.6)
Serum folate, nmol/L	13.4 (10.1-15.6)	12.8 (9.7-15.5)	13.0 (9.9-15.6)
Serum vitamin B-12, pmol/L	256 (221-335)	245 (210-290)	249 (219-303)
Energy, kJ/d	6161 (5352-7360)	5491 (4629-6729)	5820 (4976-7211)
Protein, g/d	53 (42-67)	47 (39-59)	48 (41-62)
Fat, g/d	52 (37-59)	42 (37-54)	46 (37-57)
Carbohydrate, g/d	201 (180-237)	181 (142-218)	197 (162-223)
Folate, μg/d	250 (194-356)	222 (173-279)	243 (190-296)
Folate, μg/1000 kJ	38 (33-54)	43 (35-50)	41 (34-50)
Vitamin B-12, μg/d	5.0 (2.6-6.4)	3.1 (2.6-4.7)	3.6 (2.6-5.9)
Vitamin B-6, mg/d	1.0 (0.7-1.2)	0.8 (0.6-1.0)	0.9 (0.7-1.1)
Vitamin B-2, mg/d	1.1 (0.8-1.4)	0.9 (0.8-1.2)	1.0 (0.8-1.3)
CES-D scale	10.0 (4.0-12.8)	10.5 (4.0-14.3)	10.5 (4.0-13.0)

All values are medians with 25th-75t^{h} percentiles in parentheses. Randomization groups (group A and B) did not differ in baseline characteristics except for serum tHcy (p=0.039). tHcy, serum total homocysteine; CES-D, Center for Epidemiological Studies-Depression

TABLE 1: Baseline characteristics and dietary intake of the study subjects

The study was a randomized, double-blind, crossover design conducted at a single site (Figure 1). Eligible subjects were randomly assigned in block fashion to two groups (groups A and B in Table 1). All subjects received folic acid-fortified bread and control bread in the crossover design. The initial diet period (diet period 1) was 4 weeks long, followed by an 8-wk washout period and a second 4-wk diet period (diet period 2). An 8-wk washout period is typical of feeding trials with folic acid supplementation with three genotypes of *MTHFR* [3]. The bread flour was CAMELLIA (Nisshin Seifun, Inc., Saitama, Japan) and the bread was made by SUNMERR'S, Inc. (Saitama, Japan). The folate content of the control bread was 26 μg/100 g (16 μg/slice/61.7 g bread) and that of the folic acid-fortified bread was 340 ± 21 μg/100 g (215 ± 14.7 μg/slice/64.0 g bread). Folate in the bread was determined by bioassay using *Lactobacillus rhamnous* ATCC 7469 after digestion with chicken pancreas enzyme. Subjects were given one slice a day (folate dose was 16 μg during the control period and 215 μg during the folic acid period) for 4 wk. Since the folic acid added to the bread was 215 μg of pteroylmonoglutamate, it corresponded to 344 μg dietary folate equivalents (DFE). Subjects were requested to maintain their regular dietary patterns.

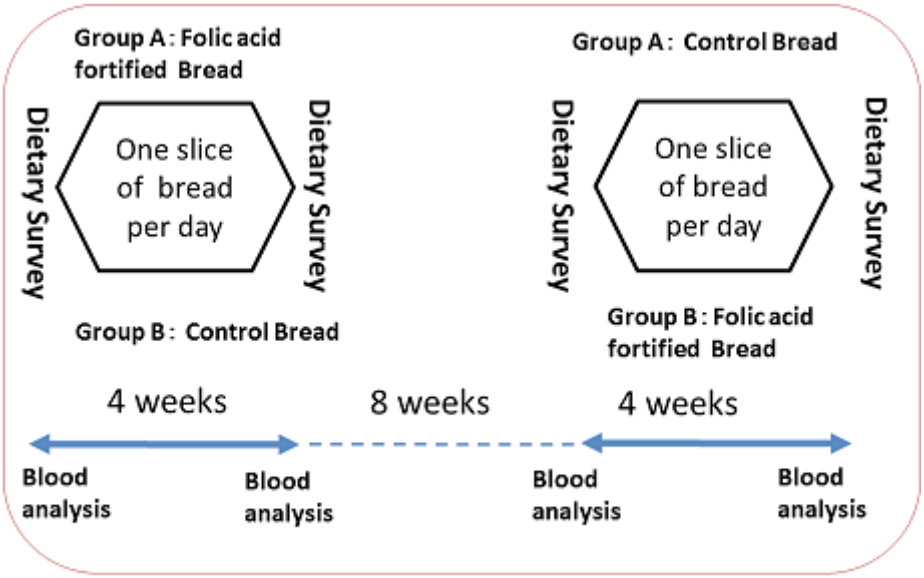

Figure 1: Illustration of the study period. Group A received folic acid fortified bread for 4 wk, underwent an 8-wk washout period, and then received control bread for 4 wk. Group B received control bread for 4 wk, underwent an 8-wk washout period, and then received folic acid fortified bread for 4 wk. Blood analysis was conducted on both groups at the start and end of both 4-wk periods.

Sample collection, genotyping and biochemical analyses

Venous blood samples were collected in plain and EDTA-containing Venoject tubes from each subject before breakfast at the beginning and end of the two diet periods, i.e., four times in total (Figure 1). Whole blood was subjected to genomic DNA extraction and purification using a MagtrationTM System device (Precision Systems Science Co. Ltd., Chiba, Japan) with magnetic particles [15]. The single nucleotide polymorphism for *MTHFR* C677T (rs1801133) was genotyped automatically using bead array in a capillary tube [16]. As described in detail in the previous report [7], polymorphisms of 5-*HTT* (SCL6A4, 5-HTTLPR) and *DRD4* exon III variable number tandem repeat (VNTR) were genotyped by the methods of Lesch *et al.* [17] and Ono et al. [18], respectively.

The serum was isolated and stored at −80 °C until analysis. Serum folate and vitamin B_{12} concentrations were measured at an external laboratory (SRL, Inc., Tokyo, Japan) using a chemiluminescence enzyme immunoassay by Access 2 (Beckman Coulter, Inc., CA, USA). The serum tHcy concentration was determined by enzyme assay using Alfressa Auto Hcy Kit (Alfressa Pharma, Inc., Osaka, Japan) [19].

Depressive symptoms assessment

Depressive symptoms were assessed using the Japanese version [20] of the CES-D [12] at the beginning and end of the two dietary periods. This scale consists of 20 questions addressing six symptoms of depression, including depressed mood, guilt or worthlessness, helplessness or hopelessness, psychomotor retardation, loss of appetite and sleep disturbance. Each question is scored on a scale of 0 to 3, and the total CES-D score ranges from 0 to 60. Depressive symptoms were defined as present when subjects had a CES-D score of 16 and over. The criterion validity of the scale has been well established both in Japan [20] and Western countries [12].

Assessment of diet

Dietary intake during the preceding month was assessed at the end of each intervention period using a validated, brief-type self-administered diet history questionnaire (BDHQ) [21], which contains questions about the consumption frequency of 56 foods and beverages commonly consumed in the Japanese population. Energy and nutrient intakes were estimated using an *ad hoc* computer algorithm for the BDHQ [21]. The validation study of the BDHQ, using 16-d weighted dietary records as a standard revealed that Pearson's correlation coefficients for folate intake in women were 0.62 [21]. Anthropometric data were determined as per the methodology in previous reports [3,7].

Statistical analyses

Data were calculated by Stat View version 5.0 (SAS Institute Inc., Cary, NC, USA) or SPSS Statistics 17.0 (IBM Japan, Ltd., Tokyo, Japan), and were presented as median and 25th-75th percentile. Because the concentrations of serum folate and tHcy were not normally distributed, logarithmic corrections were used in all statistical analyses. The effects of the folic acid intervention or control were compared with repeated-measures ANOVA with variable change from baseline, i.e., wk 4 - wk 0, as the dependent factor, and the treatment of folic acid, and the genotype as independent factor. In addition, the difference in the concentrations of serum folate and tHcy, score of CES-D between the difference genotypes of each gene were detected using Bonferroni multiple comparison. Pairs of intervention were compared using the Wilcoxon test. We established significant differences at *p* values of <0.05.

Results

Baseline characteristics of the study subjects are presented in Table 1. When comparing the groups, there were no differences in baseline characteristics except for serum tHcy concentration. At baseline (Table 1), the serum concentrations of folate, tHcy were within normal limits. Only 6.4% (n=3) of the subjects had serum folate concentrations below the lower limit, 7 nmol/L. The median of dietary folate intake was 243 μg/day, which was equal to the recommended dietary allowance (RDA) of 240 μg. In the subjects, 52.2% (n=24) of them consumed folate more than the RDA. The frequencies of *C/C, C/T* and *T/T* genotypes of *MTHFR* were 39.1%, 47.8% and 13.0%, respectively. Those of *S/S, S/L, L/L, S/XL*, and *L/XL* genotypes of 5-*HTT* were 50.0%, 39.1%, 4.3%, 2.2%, and 4.3%, respectively, where frequency of the S allele is 71%. Those of *2/4, 2/5, 4/4, 4/5, 4/6*, and *4/7* genotypes of *DRD4* were 19.6%, 4.3%, 63.0%, 8.7%, 2.2%, and 2.2%, respectively. Thus, *4/4* homozygotes were dominant among Japanese.

The 4-wk supplementation with 215 μg/d folic acid significantly increased serum folate concentration in all subjects (p<0.001, Table 2). A significant increase in serum folate was observed only in subjects with high variant frequencies in the Japanese population: *C/C* (*p*= 0.001) and *C/T* (*p*=0.001) genotypes of *MTHFR*, S/L (*p*<0.001) and *S/S* (*p*=0.002) genotypes of 5-*HTT*, and 2/4 (p=0.011) and *4/4* (*p*<0.001) genotypes of DRD4, during the folic acid period (Table 2, left).

	Genotype	n	Folic acid period			Control period			p for comparison of treatment[1]	p for comparison of treatment×genotype[1]
			0 wk	4 wk	Change Δwk 4 - 0	0 wk	4 wk	Change Δwk 4 - 0		
Serum folate, nmol/L	All	47	14.2 (10.6–17.5)	23.1 (16.4–27.8)***	8.6 (4.0–12.9)	14.4 (11.0–17.7)	13.3 (10.4–18.6)*	-1.2 (-2.8-1.0)	<0.001	
MTHFRC677T	CC	18	14.3 (10.4–21.5)	24.6 (19.1–31.1)**	10.2 (0.5–15.4)	15.5 (11.9–18.0)	16.0 (10.0–18.6)	-1.2 (-5.0–1.3)	<0.001	0.497
	CT	22	14.2 (11.1–16.1)	22.4 (16.4–25.5)**	6.9 (4.5–11.4)	14.4 (11.1–18.2)	13.1 (11.3–19.6)	-1.4 (-2.8–1.4)		
	TT	6	10.5 (8.7–18.5)	18.0 (14.0–24.5)*	5.4 (1.16–12.1)	11.3 (8.9–15.5)	10.0 (7.4–15.2)*	-1.2 (-1.5– -0.5)		
5-*HTT*	L/L	2	10.3 (6.3–9.2)	20.2 (12.4–17.8)	9.9 (3.2–11.6)	14.7 (9.9–12.2)	11.2 (7.6–9.2)	-3.5 (-4.6– -0.7)	<0.001	0.512
	L/XL	2	17.6 (10.4–16.0)	28.5 (20.2–22.6)	11.0 (4.2–12.2)	21.2 (14.8–17.0)	17.8 (11.2–15.5)	-3.4 (-3.6– -1.5)		
	S/L	18	12.7 (9.7–16.1)	21.9 (15.3–26.3)***	9.1 (5.0–12.1)	14.2 (10.0–16.06)	12.7 (9.6–16.1)	-1.4 (-2.7–1.4)		
	S/S	23	14.9 (11.1–21.1)	23.3 (16.1–28.3)**	6.8 (-0.2–12.7)	14.3 (10.6–19.9)	13.6 (10.4–19.5)	-0.9 (-3.2–1.1)		
	S/XL	1	12.5	31.5	19.0	17.4	18.3	0.9		
DRD4	2/4	9	14.9 (11.6–21.9)	25.1 (22.0–31.8)*	11.1 (8.3–13.9)	17.4 (13.3–25.5)	18.3 (12.0–19.7)	-1.4 (-6.6–0.6)	<0.001	0.011
	2/5	2	20.4 (8.3–22.3)	20.6 (12.1–18.9)	0.2 (-3.4–3.7)	12.1 (7.5–10.7)	15.7 (8.3–15.3)	3.6 (0.8–4.6)		
	4/4	29	13.8 (10.3–17.0)	22.4 (15.1–25.6)***	6.1 (1.0–10.8)	14.3 (10.5–16.6)	12.7 (9.6–18.2)	-1.1 (-2.8–1.1)		
	4/5	4	14.6 (9.9–15.1)	28.2 (24.9–29.3)	14.4 (13.0–15.4)	14.9 (11.7–19.0)	13.0 (10.8–16.8)	-1.0 (-5.1–1.0)		
	4/6	1	13.0	12.5	5.7	9.9	13.5	-1.4		
	4/7	1	11.1	25.4	14.3	14.0	11.3	-2.7		

All values are medians with 25th-75th percentiles in parentheses.
1Difference of effects of folic acid treatments and genotypes by repeated-measures ANOVA.
*, **, ***Significantly different from 0 wk: *p<0.05, **p<0.01, ***p<0.001 (Wilcoxon test).
MTHFR, methylenetetrahydrofolate reductase; 5-*HTT*, serotonin transporter; *DRD4*, dopamine D4 receptor.

Table 2: Effects of folic acid supplementation on serum folate of subjects with 14 different genotypes

During the control period, there was a slight decrease in serum folate for all subjects (p=0.022) and in subjects with the *TT* genotypes of *MTHFR* (p=0.027, Table 2, right). The changes in serum folate concentrations were significantly different between the folic acid period and control period in all subjects (p<0.001). Interactions between the response to folic acid supplementation and genotypes of *MTHFR* C677T or those of 5-*HTT* were not significant.

At 0 wk, serum tHcy concentration of *TT* homozygotes was significantly higher than that of both *C/C* and *C/T* genotypes of *MTHFR* (p<0.05, Table 3). Supplementation with folic acid during 4 wk caused a significant decrease in serum tHcy concentration (p<0.001). On the other hand, during the 4-wk control period there were no significant changes in serum tHcy concentrations. During the folic acid period, tHcy decreased significantly in subjects with the *C/C* (p=0.003) and *C/T* (p=0.002) genotypes of *MTHFR*, *S/L* (p=0.007) and *S/S* (p<0.001) genotypes of 5-*HTT*, and *4/4* (p<0.001) genotypes of *DRD4*. Although there was a small decrease in tHcy during the control period, none of genotypes showed significant change. There were significant differences between the change during folic acid period and that during control period in all subjects (p<0.001) and those with the C/C (p=0.047) and *C/T* (p=0.019) genotypes of *MTHFR*, *S/S* (p=0.022) genotypes of 5-*HTT*, and *4/4* (p=0.049) genotypes of *DRD4*. However, there were no significant changes in the serum tHcy response to folic acid supplementation within the genotypes.

	Genotype	n	Folic acid period			Control period			p for comparison of treatment[1]	p for comparison of treatment[×] genotype[1]
			0 wk	4 wk	Change Δwk 4 - 0	0 wk	4 wk	Change Δwk 4 - 0		
Serum tHcy, μmol/L	All	46	6.6 (5.6–7.5)	5.7 (4.8–6.6)***	−0.8 (-1.2– -0.3)	6.8 (5.3–7.6)	6.6 (5.4–7.3)	0.0 (-0.9–0.5)	<0.001	
MTHFR	CC	18	6.5 (5.4–7.6)†	5.5 (4.8–6.7)**	-0.8 (-1.2–0.0)	6.5 (5.2–7.1)	6.6 (5.4–7.0)	0.0 (-0.6–0.5)	<0.001	0.411
	CT	22	6.6 (5.7–7.3)†	5.9 (5.1–6.6)**	-0.8 (-1.2– -0.3)	6.8 (5.2–7.8)	6.5 (5.4–7.3)	0.0 (-0.7–0.6)		
	TT	6	6.4 (5.3–8.9)	4.6 (3.8–7.4)*	-1.3 (-2.5– -0.4)	7.4 (6.5–10.0)	6.4 (5.6–11.0)	-0.8 (-1.3–1.0)		
5-*HTT*	L/L	2	7.8 (5.6–6.1)	6.7 (4.9–5.1)	-1.1 (-1.0– -0.7)	7.3 (5.3–5.6)	7.5 (5.4–5.8)	0.2 (-0.2–0.5)	0.056	0.792
	L/XL	2	5.6 (4.0–4.4)	5.0 (3.5–3.9)	-0.6 (-0.8–0.0)	4.9 (3.4–4.0)	5.0 (3.1–1.4)	0.1 (0.0–0.1)		
	S/L	18	6.9 (5.7–7.6)	5.7 (4.8–6.7)**	-0.9 (-1.8– -0.4)	7.0 (6.3–7.8)	6.8 (5.8–7.4)	-0.1 (-1.0–0.6)		
	S/S	23	6.2 (5.4–6.9)	5.5 (4.8–6.3)***	-0.7 (-0.9– -0.3)	6.4 (5.0–7.8)	6.4 (5.4–7.4)	0.0 (-1.2–0.5)		
	S/XL	1	7.5	7.4	-0.1	6.4	6.6	0.2		
DRD4	2/4	9	6.0 (5.4–7.3)	4.8 (4.7–7.4)	-0.7 (-1.0– -0.4)	6.4 (4.2–7.5)	6.5 (4.6–7.5)	0.0 (-0.8–0.9)	<0.001	0.051
	2/5	2	6.7 (4.9–5.1)	5.7 (4.1–4.4)	-1.0 (-1.0– -0.5)	6.3 (4.4–5.1)	6.4 (4.8–4.8)	0.1 (-0.3–0.5)		
	4/4	29	6.6 (5.7–7.5)	5.8 (5.0–6.6)***	-0.9 (-1.3– -0.2)	6.8 (5.7–7.8)	6.6 (5.4–7.2)	0.0 (-1.0–0.3)		
	4/5	4	6.2 (5.5–7.8)	5.5 (4.2–6.6)	-1.1 (-1.6– -0.5)	7.1 (5.4–7.3)	6.9 (6.0–7.7)	0.4 (-0.7–1.0)		
	4/6	1	7.7	12.7	-0.5	13.4	11.3	3.6		
	4/7	1	4.6	4.0	-0.6	5.3	4.1	-1.2		

All values are medians with 25th-75th percentiles in parentheses.
1Difference of effects of folic acid treatments and genotypes by repeated-measures ANOVA.
*, **, ***Significantly different from 0 wk: *p<0.05, **p<0.01, ***p<0.001 (Wilcoxon test).
†Significantly different from *TT*: p<0.05 (Bonferroni multiple comparison).
tHcy, total homocysteine; *MTHFR*, methylenetetrahydrofolate reductase; 5-*HTT*, serotonin transporter; DRD4, dopamine D4 receptor.

Table 3: Effects of folic acid supplementation on serum total homocysteine of subjects with 14 different genotype

The CES-D score was significantly decreased during the folic acid period (all subjects, p<0.001), including *C/C* homozygotes (p=0.002) and T/T homozygotes (p=0.028), whereas it was not significantly different during the control period, except for the CC group (Table 4). CES-D scores during the folic acid period were significantly improved in the most frequent genotypes among the Japanese population: *S/S* (p=0.011) genotypes of 5-*HTT* [7] and the *4/4* (p< 0.001) genotypes of *DRD4* [7]. For the changes in CES-D score, interactions between folic acid treatment and genotypes were not statistically significant.

The characteristics of the study subjects classified by depression score (CES-D score <16) are presented in Table 5. At 0 wk of the folic acid period, 18% of the total subjects had a CES-D score 16 or more, and of the control period, 21% were the depressed subjects. During the folic acid period, serum folate increased and tHcy decreased significantly in both subjects with and without depressive symptoms, whereas during the control period, changes in the concentration of serum folate and tHcy were either insignificant or reversed.

	Genotype	n	Folic acid period			Control period			p for comparison of treatment[1]	p for comparison of treatment×genotype[1]
			0 wk	4 wk	Change Δwk 4 - 0	0 wk	4 wk	Change Δwk 4 - 0		
CES-D Score	All	46	10.0 (3.0–13.0)	5.0 (2.0–10.0)***	-2.0 (-4.0–0.0)	9.0 (4.0–13.3)	8.0 (3.0–11.3)	-1.0 (-4.0–2.0)	0.166	
MTHFR	CC	18	8.0 (3.8–15.5)	6.0 (1.8–12.5)**	-1.5 (-4.0–0.0)	12.0(7.5–15.3)	8.5 (4.5–11.8)*	-3.0 (-4.5– -1.5)	0.060	0.067
	CT	22	11.0 (2.8–12.3)	5.5 (3.0–8.0)	-1.0 (-4.3–2.0)	8.0(1.8–12.0)	8.0 (3.0–11.3)	0.0 (-1.5–2.3)		
	TT	6	9.0 (1.5–18.8)	4.5 (0.8–8.3)*	-3.5 (-10.0– -1.3)	7.0(3.0–15.3)	7.0 (1.8–15.3)	-0.5 (-5.0–5.0)		
5-*HTT*	L/L	2	10.0 (4.5–10.5)	6.0 (3.8–5.3)	-4.0 (-6.8–0.8)	12.0 (9.0–9.0)	10.0 (6.8–8.3)	-2.0 (-2.3– -0.8)	0.160	0.805
	L/XL	2	14.0 (9.8–11.3)	7.0 (2.3–8.3)	-7.0 (-7.5– -3.0)	11.0 (6.8–9.8)	9.5 (6.8–7.5)	-1.5 (-2.3–0.0)		
	S/L	18	9.5 (2.0–12.3)	6.0 (2.8–8.5)	-2.0 (-4.3–0.5)	9.5 (4.8–12.5)	8.5 (3.8–14.0)	-0.5 (-3.0–3.3)		
	S/S	23	9.0 (3.0–12.0)	5.0 (1.0–14.0)*	-2.0 (-4.0–0.0)	9.0 (1.0–15.0)	7.0 (2.0–11.0)	-1.0 (-4.0–1.0)		
	S/XL	1	5.0	5.0	0.0	8.0	5.0	-3.0		
DRD4	2/4	9	5.0 (2.0–14.0)	8.0 (2.0–14.0)	0.0 (-2.5–0.5)	5.0 (2.5–13.5)	8.0 (4.5–13.5)	0.0 (-2.5–4.5)	0.365	0.683
	2/5	2	7.0 (1.5–9.0)	8.0 (6.0–6.0)	1.0 (-3.0–4.5)	10.5 (6.8–9.0)	8.0 (6.0–6.0)	-2.5 (-3.0– -0.8)		
	4/4	29	8.0 (2.5–13.0)	5.0 (1.0–9.0)***	-2.0 (-5.0–0.0)	9.0 (4.0–13.0)	7.0 (2.0–11.5)*	-2.0 (-4.0–1.0)		
	4/5	4	11.5 (7.3–15.8)	7.5 (4.8–12.5)	-1.5 (-10.8–2.5)	13.5 (12.3–14.8)	10.0 (9.0–17.0)	-3.5 (-4.0–3.0)		
	4/6	1	11.0	6.0	-5.0	5.0	10.0	5.0		
	4/7	1	13.0	16.0	3.0	20.0	22.0	2.0		

All values are medians with 25th-75th percentiles in parentheses.
1Difference of effects of folic acid treatments and genotypes by repeated-measures ANOVA.
*, **, ***Significantly different from 0 wk: *$p<0.05$, **$p<0.01$, ***$p<0.001$.
CES-D, Center for Epidemiological Studies-Depression; *MTHFR*, methylenetetrahydrofolate reductase; 5-*HTT*, serotonin transporter; *DRD4*, dopamine D4 receptor

Table 4: Effects of folic acid supplementation on CES-D score of subjects with 14 different genotypes

		Folic acid period				**Control period**				**p for comparison of treatment[1]**	**p for comparison of score[1]**
		n	0 wk	4 wk	Change Δ wk 4 - 0	n	0 wk	4 wk	Change Δ wk 4 - 0		
Serum folate, nmol/L	<16 of CES-D score	39	13.4 (10.6–17.4)	22.4 (16.1–26.0)***	7.0 (3.2–11.8)	38	14.4 (11.1–18.2)	13.3 (10.4–18.6)*	-1.4 (-2.9–1.0)	<0.001	0.801
	≥16 of CES-D score	7	15.4 (14.3–17.7)	28.1 (21.5–31.0)*	12.7 (10.9–14.5)	8	14.4 (10.1–17.1)	14.0 (9.3–18.9)	-1.0 (-2.4–2.1)		
Serum tHcy, µmol/L	<16 of CES-D score	39	6.6 (5.4–7.5)	5.8 (4.8–6.6)***	-0.8 (-1.2–-0.2)	38	6.8 (5.7–7.6)	6.6 (5.5–7.3)	0.0 (-0.9–0.6)	<0.001	0.586
	≥16 of CES-D score	7	6.0 (5.6–7.1)	5.6 (4.2–6.6)*	-0.9 (-1.5–-0.3)	8	5.9 (5.0–8.9)	6.1 (4.3–8.0)	-0.1 (-1.0–0.2)		
CES-D score	<16 of CES-D score	39	7.0 (2.0–12.0)	5.0 (1.0–8.0)**	-2.0 (-4.0–0.0)	38	8.0 (3.8–12.0)	7.0 (2.8–9.3)	-1.0 (-3.3–1.3)	0.088	0.007
	≥16 of CES-D score	7	20.0 (17.0–23.0)	17.0 (15.0–19.0)	-3.0 (-9.0–0.0)	8	19.5 (17.3–27.5)	19.0 (14.0–23.8)	-1.5 (-4.8–2.8)		

Energy intake, kJ/d	<16 of CES-D score	39	6158 (5135–7237)	6309 (4909–7435)	152 (-726–1064)	38	5769 (5071–6965)	6174 (4946–7049)	212 (-619–766)	0.674	0.012
	≥16 of CES-D score	7	5781 (3194-5877)	6071 (3617-5877)	423 (-1002–723)	8	4797 (6223–7038)	5652 (4450–6801)	-3 (-39–46)		
Folate, µg/d	<16 of CES-D score	39	255 (194–355)	274 (201–335)	14 (-42–57)	38	229 (184–329)	249 (172–361)	16 (-27–67)	0.405	0.471
	≥16 of CES-D score	7	184 (139–233)	229 (149–271)	10 (-10–62)	8	206 (172–252)	200 (153–333)	-3(-39-46)		

All values are medians with 25th-75th percentiles in parentheses.
[1]Difference of effects of folic acid treatments and CES-D score (<16 and ≥16) by ANOVA.
*, **, ***Significantly different from 0 wk: *p<0.05, **p<0.01, ***p<0.001.
tHcy, total homocysteine; CES-D, Center for Epidemiological Studies-Depression.

Table 5: Effects of folic acid supplementation on serum folate, serum total homocysteine and CES-D scores of subjects without depressive symptoms (CES-D score ≥16) and subjects without depressive symptoms (CES-D score <16).

The reduction in the CES-D score for the subjects without depressive symptoms was significant (p=0.002) and that in the depressed subjects was marginal (p=0.059) during the folic acid period, but the reduction of CES-D in both groups was insignificant during the control period (Table 5). In order to rule out the possibility that folate deficiency is a consequence of depression rather than its cause, as depression reduces appetite, both baseline energy and folate intake were compared. The changes in baseline intake for both groups were not significant for either test period. There were no differences in food group and nutrient intake except folate calculated from BDHQ [21] revealed significant correlation with CES-D score. With the exception of serum folate and tHcy, none of the serum components, including albumin, lipids and glucose, were significantly correlated with CES-D score (data not shown). There were no important adverse events or side effects of folic acid supplementation tested by detailed blood biochemistry and health check. We previously examined five other single nucleotide polymorphisms related to C-1 metabolism, but only the *T/T* genotype of *MTHFR* significantly affected serum concentrations of both folate and tHcy (n=250; p<0.005) [3].

Discussion

This study assesses the relationship among folate status, depression-prone genetic polymorphism [2-10] and depressive symptoms in Japanese subjects, whose genetic background increases susceptibility to depression more than that of Caucasians [4-10]; the prevalence of *T/T* homozygotes of *MTHFR* is about 2-fold [1,3], that of S/S homozygotes of 5-*HTT* is about 4-fold [7,8] and that of *4/4* homozygotes of *DRD4* is 1.2-fold higher among Japanese [3,10] than Caucasians. In Japan, the prevalence of depressive symptoms is 36.1% for men and 36.4% for women, if depressive symptoms are defined as a CES-D score of 16 or more [11]. However, in a Rotterdam study, prevalence of depression was only 7.1% (278/3884) using the same criterion for depressive symptoms (CES-D score ≥16) [22]. The prevalence of depressive symptoms in this study was 18-21% (Table 5). Since gene-environment interactions in the etiology of depression is evident [1,6,14], the high prevalence of depressive symptoms among Japanese [11,23] may be caused by both the genetic background [1,3,7,8] and the low folate status [1,11,14,23] (Table 2). In Japanese women, folate intake is from 235 µg/d for 20- to 29-year-olds to 330 µg/d for 60- to 69-year-olds [24], and serum folate ranges from 18.1 nmol/L for 21-year-olds [3] to 27.5 nmol/L for 66-year-olds [3]. These values are lower than those of American women, which are 680 µg/d = 994 µg DFE/d for 55-year-olds [25] and 34.2 nmol/L for 70-year-olds [26], respectively. The difference in folate status of both countries is caused by the compulsory folic acid fortification of cereals in the U.S., not in Japan.

The nutrients described in Table 1, i.e., folate, vitamins B_2, B_6, and B_{12}, and protein, are required for homocysteine metabolism and S-adenosylmethionine serves as the universal methyl donor in a variety of methylation reactions, including those involving neurotransmitters and epigenetic modification of DNA [1,14].

Table 1 indicates that the baseline median daily intakes of folate, vitamins B_2, B_6, B_{12} and protein were close to the 2015 RDA for Japanese women [18-29 years]; RDAs are 240 µg, 1.2 mg, 1.2 mg, 2.4 µg, and 50 g, respectively [27]. The baseline folate intake in Table 1 was close to the average intake of folate in Japanese women (235 µg/d) [24]. However, CES-D scores of subjects tended to improve by taking 215 µg/d more folic acid, i.e., the total folate intake during the folic acid period was 455 (240 from diet + 215 from folic acid) µg/d; 605 µg DFE/d. In the U.S., the adjusted odds ratio for high CES-D score in women in the lowest tertile of folate (plasma folate: 5.26-25.4 nmol/L) was 2.04 compared with that in women in the highest tertile of folate (p<0.001) [26]. A cross-sectional observational study in Japanese subjects revealed that folate intake had a statistically significant, inverse and linear

association with depressive symptoms in men but not in women [11,23]. However, this interventional study showed improvement of depression symptoms in young women. This gender difference of folate status in depressive symptoms was compared using the CES-D score [28]. Compared with the lowest tertile, the middle and uppermost tertiles of plasma folate were associated with a 39-40% reduced odds of elevated CES-D score (>16) among women (p=0.006) [28]. On the other hand, by structural equation modeling, plasma folate completely mediated the inverse healthy eating index-CES-D association among men only [28].

Meta-analysis of psychiatric tests including CES-D [12] indicates that the polymorphisms of *MTHFR* [2,4,5], 5-*HTT* [6,10,29] and *DRD4* [9,10] have a small but reliable influence on depression and personality [29]. Serotonin transporter inhibitor is an effective antidepressant and S/S homozygote of 5-*HTT* is a risk factor for depression [6]. In fact, functional magnetic resonance imaging (fMRI) illustrated greater amygdala activity in S-allele carriers in comparison with *L/L* homozygotes of 5-*HTT*, because the amygdala response is crucial for depression-related temperamental traits [8,30]. In addition, the imaging by single-photon emission computed tomography (SPECT) of midbrain serotonin transporter revealed lower midbrain radioactive ligand binding of the depression patients compared with the controls [31]. The behavioral phenotypes may be affected by environmental factors including stress [6] and folate status [1,2,13,14] (Table 2). In addition to the CES-D score, magnetic resonance imaging (MRI) revealed that white-matter hyperintensities had significant correlations with both tHcy and depressive symptoms in patients with low folate levels [32]. Although the elucidation of brain imaging and molecular mechanisms of depression is strongly needed, clinical supplementation of folate to reduce depressive symptoms is possible. In support of this study, clinical folate supplementation in the treatment of depressive patients with [14,33] or without antidepressants [14,34] has been partially successful. Folate has been found to further reduce symptoms in folate-deficient patients with depression when used in conjunction with an antidepressant [33]. In a prospective, double-blind, placebo-controlled study, hospitalized older patients received either a normal hospital diet plus nutritional supplements providing 12 vitamins and 11 minerals or a normal hospital diet plus a placebo daily for 6 months [34]. The supplement significantly increased red cell folate and reduced depression symptoms in all patient groups including those with depression (p=0.021) [34].

This study had some limitations. First, although the narrow age range (18-26 years, median 20 years) of women makes the sample more homogeneous, we were unable to examine the effects of the predictor variables in older individuals and men [1,2,20]. In a previous folic acid supplementation experiment without CES-D testing, we confirmed similar effects of fortified folic acid bread on serum folate (p<0.001) and tHcy (p<0.001) in male subjects (n=30) with all genotypes of *MTHFR* (unpublished data). A second limitation was the lack of a mechanistic study in the brain between folate input and CES-D score output. In order to elucidate, functional MRI (fMRI) tests of the amygdala [8,30] and single photon emission computed tomography (SPECT) tests of serotonin in brain [31], to substantiate CES-D scoring [12], are needed. A third limitation was the lack of combination data of 14 genotypes of *MTHFR*, 5-*HTT*, and *DRD4* because of low statistical power caused by the small sample number and rare variant frequencies, thus a large study sample is needed. A fourth limitation was the omission of other candidate genes for depression including the *ANK3* (ankyrin3) gene found by the psychiatric genome wide association studies (GWAS) [35].

Nutritional inadequacy of folate is widespread; hence, programs to increase supplemental intakes of 100 μg folate/d have been recommended [36], but an adequate amount of folate depends on genetic polymorphisms [3]. In fact, in *T/T* homozygotes of *MTHFR* the serum folate was lower and tHcy was higher at 0 wk of the folic acid period compared with other genotypes (Table 2, 3). Nutritional intervention with folate may be a cost effective way of preventing and treating depression and other psychosis including schizophrenia [37]. To improve depressive symptoms and folate status of young Japanese women with genetic polymorphisms vulnerable to depression, folate intake greater than the RDA may be necessary.

Conclusion

Folic acid supplementation in Japanese women with depression-related polymorphisms was effective in reducing depression scores.

Acknowledgment

We thank the 46 participants in the dietary intervention for their cooperation. The authors' responsibilities were as follows: MH was responsible for recruiting subjects, conceived and designed the study, and participated in the statistical analysis. AF and KS genotyped genes for 5-*HTT* and *DRD4*. HM and HS designed the research, and YS provided the essential reagents. RN and YJ assisted in the psychiatric analysis, organization of subjects and nutritional survey. YK participated in the total planning of this trial, interpreted the data, supervised the acquisition of blood samples as a medical doctor, and was responsible for drafting the article. The authors report no conflicts of interest with respect to this study.

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